REMARKS

Status of the claims

Claims 21 and 37-43 are pending and claims 21 and 41-43 are under consideration in this application, claims 37-40 having been withdrawn for allegedly being drawn to a separate invention. All the claims under consideration stand rejected.

For the sake of consistency, the term "early stage" in claims 37, 38, 39, and 40 has been replaced with "early-stage". In addition, a typographic error in claim 21 is corrected. These amendments add no new matter.

From the comments on page 2, line 5, to page 3, line 14, of the Office Action, Applicants understand the Examiner's position to be that claims 37-40 are drawn to a separate invention because the test samples specified by these claims are blood rather than urine and because the claims specify the absence of symptoms not required by claim 21. While not necessarily agreeing with this position, in order to expedite prosecution of the instant application, Applicants have amended claims 37-40 by changing the test bodily fluids from blood to urine. These amendments, which are supported by the specification (e.g., at page 9, lines 19-25 and page 10, lines 6-10), add no new matter. In addition, Applicants agree that claim 21 does not require that test subjects lack the symptoms that claims 37-40 specify test subjects to lack. However, claim 21 is broader than, and overarches, claims 37-40 in that the test subjects it specifies are not excluded from lacking, and could indeed lack, the relevant symptoms. In light of these considerations, Applicants respectfully request that the restriction requirement be withdrawn and that claims 37-40 be considered in the instant application.

Provided that the restriction requirement is withdrawn, after entry of the amendments made herein, claims 21 and 37 – 43 will be pending and under consideration in this application.

35 U.S.C. § 112, second paragraph, rejections

Claims 41 and 43 stand rejected as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter that Applicants regard as the invention.

With respect to the comments on page 4, lines 1-3 of the Office Action, Applicants have amended claim 41 by replacing the term "body fluid sample" with the term "urine sample from the test subject." This amendment, which is supported by the specification (e.g., at page 10, lines 6-10) and adds no new matter, renders the rejection moot.

With respect to the comments on page 4, lines 4-10, of the Office Action, Applicants point out that claim 43 is not dependent on claim 21 and thus does not contradict it. Indeed, in the claims on which claim 43 is dependent (i.e., claims 37-40), the reference sample was, as in claim 43, blood. However, Applicants have, as described above, amended claims 37 –40 as well as claim 43 to specify that the reference sample, like the test samples, is urine. In light of these considerations, Applicants respectfully submit that the rejection is moot.

35 U.S.C. § 103(a) rejection

Claims 21, 41 and 42 stand rejected as allegedly being unpatentable over Hoffmann et al. Applicants respectfully traverse the rejection.

From the comments on page 5, line 13, to page 7, line 20, of the Office Action, Applicants understand the Examiner's position be that Hoffmann et al. renders the present claims obvious because it discloses that: (a) L-PGDS "accumulates more significantly in serum in pathological conditions than other proteins in current use" (page 6, lines 2-3, of the Office Action); (b) L-PGDS "may be used for the study and early diagnosis of renal diseases" (page 6, lines 4-5, of the Office Action); (c) that L-PGDS can be detected in urine and that in renal diseases elimination of proteins through the kidney is disturbed with the result that the protein concentration is elevated (e.g., at page 7, lines 8-11, of the Office Action); and (d) the L-PGDS in serum is identical to the L-PGDS in urine (e.g., at page 7, lines 12-13, of the Office Action). Applicants disagree with this position.

With reference to the comments on page 6, line 22, to page 7, line 7, of the Office Action, Applicants point out that their intention in quoting Hirawa et al. was only to emphasize the fact that blood and urine levels of a substance are not always proportionately correlated.

In even stronger support of this concept, Applicants enclose copies (as Exhibit A) of three references that describe the excretion patterns of heart fatty acid-binding protein (H-FABP) (Sohmiya et al. J. Mol. Cardiol. 25:1413-1426, 1993; Górski et al. Clin. Chem. 43(1): 193-195, 1997; and Hayashida et al. J. Cardiovasc. Surg. 42:735-740, 2001).

Sohmiya et al. describes the elimination of H-FABP by the kidney (see, for example, the Abstract and page 1419, column 1, line 1, to page 1423, column 1, line 2).

Górski et al. describes increased levels of H-FABP in the plasma of patients with chronic renal disease (see, e.g., Table 1).

Hayashida et al. describes the discordance between serum and H-FABP levels in patients undergoing primary coronary bypass grafting and varying levels of renal function (see, e.g., the Abstract). The patients described in the study were classified by preoperative renal function into two groups: a control group (n = 12) consisting of patients with creatinine clearances (Ccr) of 40 mL/min or greater; and a renal dysfunction group (n=7) consisting of patients with Ccr of less than 40 mL/min (see, e.g., page 736, column 1, lines 19-24). Before operation, serum CK-MB (MB isoenzyme of creatine kinase) and troponin-T levels and urinary H-FAHB excretion rate did not differ between the groups but serum H-FABP levels were significantly higher in the renal dysfunction group than in the control group (e.g., page 737, column 2, lines 38-42, and Fig. 1).

In addition, the H-FABP levels in serum increased and reached peak levels one hour after aortic declamping in the control group but the increase persisted until 18 hours after in aortic declamping in the renal dysfunction group. Moreover, the serum H-FABP levels were significantly higher 1, 2, 3, 5, and 18 hours after aortic declamping in the renal dysfunction group than in the control. On the other hand, the urinary H-FABP excretion rate was significantly lower in the renal dysfunction group 30 minutes, and 1 and 2 hours after aortic declamping (e.g., page 738, column 2, lines 5-17, page 739, column 1, lines 1-2, and Fig. 1).

In summary, the findings of Hayashida et al. are that the levels in serum of a substance (H-FABP) in preoperative and perioperative patients having renal disease are higher than in patients without renal disease. On the other hand, urinary levels of the substance either did not

differ between patients with and patients without renal disease or were actually decreased in the patients with renal disease relative to patients without renal disease.

The above findings reinforce Applicants' argument that findings made in serum cannot, without appropriate experimentation, be extrapolated to other bodily fluids such as urine. Thus, in view of the above consideration, the above-described disclosure of Hoffmann et al. in regard to L-PGDS by no means suggests, or would cause one of ordinary skill in the art to believe, that increased urinary L-PGDS levels are diagnostic of any kidney disease, let alone early kidney disease. Indeed, it was the findings of the present inventors, which were disclosed for the first time in the priority document of the instant application, that first taught that increased urinary levels of L-PGDS are indeed diagnostic of early kidney disease.

In addition, in regard to detection of renal disease by analyzing urine, the unexpected advantages over methods involving serum or plasma analysis provide further evidence of the non-obviousness of the instant claims. For example, Figure 5 of the present application shows the correlations between serum L-PGDS and creatinine concentrations and the progression of diabetes (Figure 5A) and the correlations between urinary L-PGDS index and urinary albumin index and the progression of diabetes (Figure 5B). Renal dysfunction is a frequent symptom of diabetes. The L-PGDS concentration in the serum of subjects who have had diabetes for less than five years is almost the same as that of control healthy subjects. On the other hand, the L-PGDS index of urine from subjects who have had diabetes for less than five years is significantly less than that of control healthy subjects. Thus, urine analysis is surprisingly more sensitive than serum analysis at detecting early-stage renal disease.

Moreover, these data presented in the present application provide enabling support for the detection of early-stage renal disease using urine from test subjects where the concentration of creatinine in the serum of the test subjects is normal (claim 37 and claim 40), the subjects do not demonstrate proteinuria (claim 38 and claim 40), and/or the concentration of albumin in the urine of the test subjects is normal (claim 39 and 40).

Hoffmann et al. discloses that the level of L-PGDS in serum increases in dialysis patients, i.e., patient with advanced-stage renal disease. Even though Hoffmann et al. suggests the

correlation between serum L-PGDS level and early renal disease, it does not provide specific parameters of early renal disease. Thus, Hoffmann does not disclose, or even suggest, that the L-PGDS level in urine, in addition to that in serum, increases in early renal disease, the serum concentration of creatinine being normal, the test subject not demonstrating proteinuria, or the concentration of albumin in the urine of the test subject being normal. Tables 1 and 2 of the present application show the results of a follow-up survey of diabetic patients and outpatients, respectively. The serum L-PGDS levels, urinary L-PGDS levels, serum creatinine levels, and urinary albumin levels of fourteen patients were measured at the beginning of the study. At the beginning time point, serum creatinine and urinary albumin levels were normal in all fourteen patients. However, L-PGDS concentrations and/or urinary L-PGDS indices were abnormal. Measurements made two years later showed that nine of the fourteen patients had abnormal serum creatinine and urinary albumin levels; these nine patients were diagnosed as having renal disease. These results show that the urine-based method of claims specifying analysis of urine L-PGDS levels where the concentration of serum creatinine, is normal, the concentration of urinary albumin is normal, and the test subject does not exhibit proteinuria, like the methods of claims without the latter three limitations, have unexpected advantages of over serum- or plasma-based methods.

In light of the above considerations, Applicants respectfully request that the rejection under 35 U.S.C. § 103(a) be withdrawn.

CONCLUSION

In summary, for the reasons set forth above, Applicants maintain that the pending claims patentably define the invention. Applicants request that the Examiner reconsider the rejections as set forth in the Office Action, and permit the pending claims to pass to allowance.

If the Examiner would like to discuss any of the issues raised in the Office Action, Applicants' undersigned representative can be reached at the telephone number listed below.

Enclosed is a request for an automatic extension of time and a check in payment of the extension in time. Please apply any other charges or credits to Deposit Account No. 06-1050, referencing Attorney Docket No. 11283-009001.

Respectfully submitted,

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Plasma and Urinary Heart-Type Cytoplasmic Fatty Acid-Binding Protein in Coronary Occlusion and Reperfusion Induced Myocardial Injury Model

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K. Sohmiya, T. Tanaka, R. Tsuji, K. Yoshimoto, Y. Nakayama, Y. Hirota, K. Kawamura, Y. Matsunaga, S. Nishimura and H. Miyazaki. Plasma and Urinary Heart-Type Cytoplasmic Fatty Acid-Binding Protein in Coronary Occlusion and Reperfusion Induced Myocardial Injury Model. *Journal of Molecular and Cellular Cardiology* (1993) 25, 1413–1426. The leakage of heart-type cytoplasmic fatty acid-binding protein (H-FABP$_c$) from injured myocardial cells has been reported. We have previously proposed that its plasma and urinary levels could be used as an early indicator of myocardial injury and also reflect the severity of myocardial injury. To confirm this hypothesis, the time course of changes of the plasma and urinary H-FABP$_c$ was investigated during myocardial injury induced by coronary artery occlusion and reperfusion in dogs. The plasma elimination kinetics and urinary excretion kinetics of H-FABP$_c$ were also analysed in dogs which were given a bolus injection of exogenous H-FABP$_c$. The distribution of circulating H-FABP$_c$ was determined in mice by whole-body autoradiography using ^{125}I-labelled H-FABP$_c$.

In myocardial injury model, plasma and urinary H-FABP$_c$ level showed rapid increase after reperfusion. The elimination kinetic study revealed that H-FABP$_c$ was mono-exponentially cleared from the circulation. The elimination rate constant (Ke) was 0.0275 ± 0.0094/min (mean $\pm$ S.D., $n=7$) and the disappearance half-time ($t_{1/2}$) was 27.5 ± 8.4 min (mean $\pm$ S.D., $n=7$). Exogenous H-FABP$_c$ appeared in urine soon after administration, with the peak level being at 6.9 ± 2.0 min (mean $\pm$ S.D., $n=7$). Whole-body autoradiography also demonstrated that ^{125}I-H-FABP$_c$ accumulated rapidly in the kidneys.

This study demonstrated that H-FABP$_c$ leaked rapidly from injured myocardium and rapidly appeared in plasma and urine. Infarct size was closely correlated with the calculated H-FABP$_c$ release ($r=0.89$, $r^2=0.80$, $P<0.01$, $n=7$) and with the amount of the urinary H-FABP$_c$ ($r=0.94$, $r^2=0.88$, $P<0.01$, $n=7$). These data suggest that measurement of the H-FABP$_c$ levels in plasma and urine might be useful for the early detection of myocardial injury and also for the assessment of infarct size.

Key Words: Heart-type cytoplasmic fatty acid-binding protein (H-FABP$_c$); Myocardial injury indicator; Circulatory and excretory kinetics.

Introduction

It has been clearly established that mortality and morbidity in acute myocardial infarction (AMI) are dependent on infarct size, and that early recanalization of the occluded coronary artery improves left ventricular function and reduces mortality (Braunwald, 1989; Simoon *et al.*, 1986; van de Werf and Arnold, 1988). Recanalization therapy is now widely accepted as effective for the salvage of jeopardized myocardium, so early diagnosis and accurate estimation of infarct size are indispensable parts of the management strategy. However, an accurate diagnosis of AMI in the hyperacute phase is often difficult because chest pain is frequently atypical, electrocardiographic (ECG) changes are equivocal, and the elevation of plasma cardiospecific enzymes only occurs some hours after the onset.

It has been reported that heart-type cytoplasmic fatty acid-binding protein (H-FABP$_c$) leaks from injured myocardium and may be

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useful as an indicator of myocardial damage (Glatz *et al.*, 1988; Knowlton *et al.*, 1989a, 1989b; Kleine *et al.*, 1992). We have previously reported that H-FABP$_c$ was detectable in the plasma and urine shortly after the onset of AMI (Tanaka *et al.*, 1991) and that serial measurement of the plasma H-FABP$_c$ was useful for the non-invasive detection of successful revascularization (Abe *et al.*, 1991). H-FABP$_c$ has a low molecular weight (14–16 kDa) and a cytosolic localization, so it would be expected to easily leak out from the injured myocardial cells, to be eliminated rapidly from the circulation, and to appear rapidly in the urine. However, the precise elimination and excretion kinetics of this protein have not been determined in both normal and pathological conditions. These data are necessary to know before H-FABP$_c$ can be used clinically as an indicator of myocardial injury. Moreover, by knowing its elimination kinetic from the circulation, H-FABP$_c$ might be applied for an estimation of infarct size, as same as creatine kinase and myoglobin (Shell *et al.*, 1971; Norris *et al.*, 1975; Groth *et al.*, 1987).

Urinary indicators of myocardial injury are almost unknown and only myoglobin has been tried to use as an urinary myocardial injury indicator. (Adams and Elliot, 1970; Levine *et al.*, 1971; Saranchak and Bernstein, 1974; Kessler *et al.*, 1975; Donald *et al.*, 1977). Moreover, the estimation of infarct size with an urinary substance has never been attempted. However, if the urinary H-FABP$_c$ content reflects the amount of H-FABP$_c$ leaked from injured myocardium, it may be possible to assess the severity of myocardial damage by measuring the urinary H-FABP$_c$.

In order to clarify these points, acute myocardial injury was produced in dogs by coronary occlusion-reperfusion and the time course of changes in the plasma and urinary H-FABP$_c$ was analysed. The circulation and excretion kinetics were also determined by injecting exogenous H-FABP$_c$ into closed-chest dogs. Furthermore, the distribution of circulating H-FABP$_c$ was examined by whole-body autoradiography in mice using ^{125}I-labelled canine H-FABP$_c$.

We discuss here the usefulness of the plasma and urinary H-FABP$_c$ as an early indicator of myocardial injury and the possible application for the assessment of infarct size.

Materials and Methods

Purification and competitive enzyme immunoassay (C-EIA) of H-FABP$_c$

The previously produced monospecific anti-human H-FABP$_c$ rabbit antibodies (Tanaka *et al.*, 1991) were found to have no strong reactivity with canine H-FABP$_c$. Therefore, purified canine H-FABP$_c$, and monospecific anti-canine H-FABP$_c$ rabbit antibodies were prepared for this experiment.

Purification of H-FABP$_c$ from canine hearts was performed according to the method described previously (Tanaka *et al.*, 1991). The protein content was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as the standard (Tanaka *et al.*, 1979). Monospecific polyclonal anti-canine H-FABP$_c$ antibodies were prepared in Japanese white rabbits, and the competitive enzyme immunoassay (C-EIA) for canine H-FABP$_c$ was performed in the same manner as that for human H-FABP$_c$ (Tanaka *et al.*, 1991).

Surgical procedures

Fifteen female mongrel dogs weighing 8.5–12.0 kg were anaesthetized with intravenous sodium pentobarbital (35 mg/kg) and anaesthesia was maintained with additional doses as necessary. The animals were intubated and ventilated with room air supplemented with a low flow of oxygen, so that blood gases were kept within the physiological range. A standard 12-lead electrocardiogram was monitored. Catheters were inserted into the right femoral vein for administration of fluids or drugs, and into the right femoral artery for blood pressure monitoring and blood sampling. An 8-French Foley catheter was placed in the bladder for urine collection. Thoracotomy was performed at the fourth or fifth left intercostal space and the pericardium was opened. The left anterior descending coronary artery (LAD) was carefully dissected out just distal to the first or second diagonal branch. The animals were allowed to stabilize for at least 1 h before the subsequent procedures. After intravenous administration of 5000 units of

heparin and 20 mg/kg aspirin, the LAD was gently ligated with a silicone tube for a 2-h period, and then released by removal of the tube. Fifty milligrams of lidocaine was injected intravenously just prior to occlusion and release of the LAD.

In two control dogs, the LAD was dissected out in the same manner but was not occluded.

There was no evidence of renal impairment in any of the dogs on the basis of the plasma creatinine level and the electrolyte concentrations.

Blood and urine sampling

Blood (2 ml) and urine samples were obtained just before LAD ligation for the measurement of baseline H-FABP$_c$ and creatine kinase (CK) activity. During 2-h occlusion, blood and urine samples were collected at 30 min intervals. For the first 30 min after LAD release, blood samples were obtained at 5 min intervals and urine samples at 15 min intervals. Subsequently, both blood and urine samples were taken at 15 min intervals for the next 90 min, at 30 min intervals from 2 to 6 h, and at 60 min intervals until the end of the experiment. The urine volume collected during each interval was measured and presented as urine volume per minute (l/min).

Measurement of H-FABP$_c$ level and CK activity

The H-FABP$_c$ level (μg/l) in plasma and urine was determined by C-EIA. Samples which contained high levels of H-FABP$_c$ were diluted 10- to 20-fold with phosphate buffered saline. Plasma CK activity was measured using a kit obtained from Shinotest Corporation (Tokyo, Japan). Urinary flow varied over a wide range both intra-experimentally and inter-experimentally in each dog. Urinary H-FABP$_c$ levels also varied over a wide range, depending on time-to-time urinary flow variation. Then in the study of coronary occlusion and reperfusion induced myocardial injury, urinary H-FABP$_c$ was expressed as excretion rate; H-FABP$_c$ excretion rate (ng/min) = urinary H-FABP$_c$ level (μg/l) × urine volume per minute (l/min) × 1000. Measurements were performed in duplicate or triplicate. The results varied by less than 5% and the mean values were determined.

H-FABP$_c$ and CK release were calculated according to the formula of Shell *et al.* (1971), i.e., H-FABP$_c$ (or CK) release $= E + Kd\Sigma Edt$, where E is the plasma H-FABP$_c$ (or CK) level at time t minus the baseline plasma level, t is the time after coronary ligation, and Kd is the fractional H-FABP$_c$ (or CK) disappearance rate. Kd values were calculated from the concentration-time curve obtained in each dog as described by Norris *et al.* (1975).

Determination of infarct size

After termination of the experiment, the dogs were killed with a high dose of potassium chloride and their hearts were quickly removed. Each heart was cut transversely into six or seven slices from the apex to the base and the extent of myocardial infarction was determined by triphenyl tetrazolium chloride (TTC) staining (Lie *et al.*, 1975). After weighing, both surfaces of each slice were photographed in colour and traced onto white paper with the help of a slide projector. The total cross-sectional area and the infarct area were measured using a planimeter. Then the weight of the infarcted myocardium was calculated as follows: infarct weight (g) = Sigma (Σ) (% infarct area on the upper surface + % infarct area on the lower surface)/2 × the slice weight (g).

Kinetic studies of H-FABP$_c$

An investigation of the disappearance of H-FABP$_c$ from the circulation and its appearance in the urine was performed in seven female mongrel dogs weighing 8.0–14.0 kg by following the administration of exogenous H-FABP$_c$. All the animals had normal renal function, as confirmed by the plasma creatinine and electrolyte levels. Anaesthesia and ventilation were same as described in Surgical procedures. Thoracotomy was not performed. A catheter was introduced into the right atrium and a second catheter was introduced into the femoral vein. A third catheter was inserted into the femoral artery for blood pressure monitoring and blood sampling. A saline solution containing 2 units/ml of heparin was infused via the second catheter

until the end of the experiment. Urine was drained from the bladder with an 8-French Foley catheter. A solution containing purified H-FABP$_c$ at a mean dose of 22.0 μg/kg (range: 16.6–27.4 μg/kg) was administered rapidly into the right atrium. Blood (1 ml) and urine samples were collected at 2-min intervals for 30 min after administration and at 3-min intervals from 30 to 60 min. Plasma and urinary H-FABP$_c$ was measured by C-EIA as described in Measurement of H-FABP$_c$ level and CK activity. The recovery of H-FABP$_c$ into the urine was calculated using the following formula: urinary H-FABP$_c$ (μg/l) × urine volume (l)/dose of H-FABP$_c$ administered to each animal (μg) × 100.

The elimination curve of exogenous H-FABP$_c$ was analysed by fitting the data to a one- or two-compartment model using a MULTI computer program (Yamaoka *et al.*, 1981), and the fit was judged by Akaike's information criterion (AIC) (Yamaoka *et al.*, 1978).

Whole-body autoradiography with ^{125}I-labelled H-FABP$_c$

Purified H-FABP$_c$ was labelled with ^{125}I using the chloramine T method according to the procedure described by Hunter and Greenwood (1962). A specific activity of 205.7 μCi/mg was obtained. Whole-body autoradiography was performed after intravenous administration of 0.216 μg/kg of ^{125}I-H-FABP$_c$ in mice. Whole-body sections of 40 μm in thickness were cut using a cryostat, freeze-dried, and placed in contact with Kodak X-ray SB film. Some freeze-dried sections were treated with ice-cold 5% perchloric acid to remove acid-soluble radioactive materials, as described previously (Kim *et al.*, 1987).

Statistical analysis

Data were expressed as the mean ± S.D. and statistical analysis was performed using ANOVA. Linear regression slopes were tested for significant differences using the *t*-test.

Results

Purification and C-EIA of H-FABP$_c$

The H-FABP$_c$ prepared from canine hearts appeared to be homogenous when analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE).

H-FABP$_c$ levels ranging from 5 to 250 ng were measured reproducibly by the C-EIA. The within-day and between-day coefficients of variation for the assay, and recovery tests using plasma and urine were essentially the same as those described previously (Tanaka *et al.*, 1991).

Surgical procedures

Myocardial infarction was induced in seven of the 15 dogs. Infarction was confirmed both by sustained ST segment elevation on the electrocardiogram and by TTC staining. Three of the 15 animals died of ventricular fibrillation soon after LAD occlusion and myocardial infarction was not produced in five dogs, as judged by the ECG changes and TTC staining. Data from the seven dogs with confirmed myocardial infarction was used in this study.

TTC staining and measurement of infarct size

Non-infarcted myocardium stained red by TTC because of the action of a dehydrogenase enzyme, whereas the infarcted area was not stained and remained pale. Thus, the infarcted area was easily distinguished from the surrounding normal myocardium by this method. The infarcted region was localized on the endomyocardial side of the anterior wall (Fig. 1) and ranged from 2.1 to 6.2 g in weight, which corresponded to from 7.1 to 16.9% of the ventricular weight.

Changes of plasma H-FABP$_c$ and CK levels and urinary H-FABP$_c$ excretion

Plasma H-FABP$_c$ level before ligation was 15.3 ± 5.5 μg/l and the urinary excretion was 19.6 ± 10.8 ng/min (urinary H-FABP$_c$ levels were from 19.4 ± 6.2 μg/l during the 15 min before ligation). Plasma CK activity was 263 ± 178 U/l. After reperfusion, plasma H-FABP$_c$ levels rose markedly and reached a

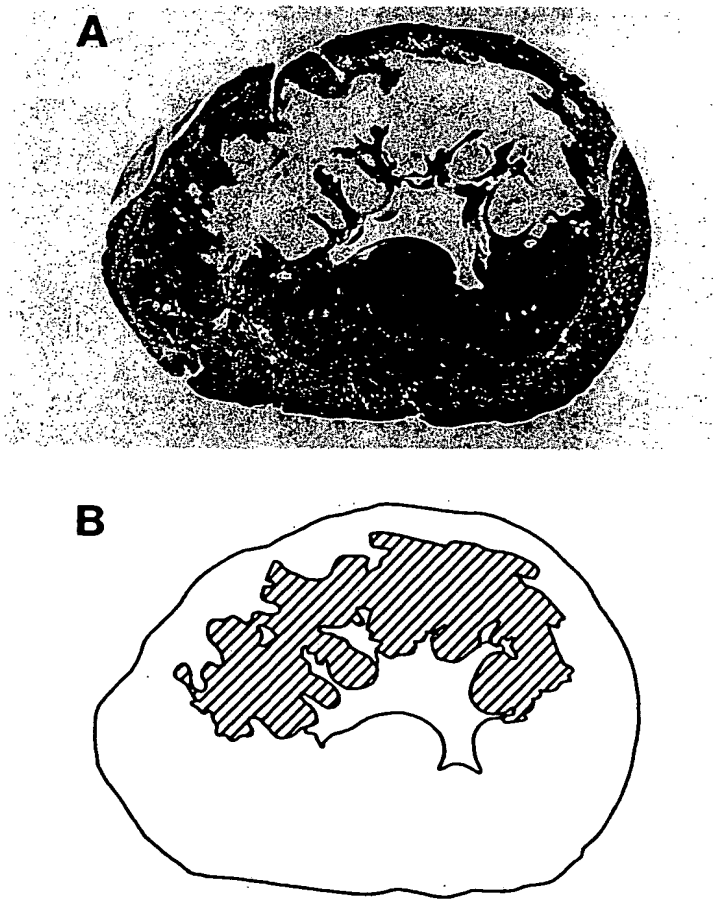

FIGURE 1. A: Photograph of a cardiac section stained with TTC. The infarcted area is pale (white) and is clearly demarcated from the normal myocardium, which is stained red (dark). B: The infarcted area (shaded) was traced and measured using a planimeter. Then infarct size was calculated as described in Materials and Methods.

peak (188.8 ± 31.8 μg/l) by 20–45 min. In contrast, plasma CK activity reached its maximal level (7223 ± 2375 U/l) after 105–270 min. The time until the peak plasma H-FABP$_c$ level was significantly shorter than that for CK (27 ± 9 min *vs* 174 ± 47 min, $P < 0.01$, Table 1). Excretion of H-FABP$_c$ in the urine occurred rapidly and reached its peak (4196 ± 1359 ng/min) at 45–75 min after reperfusion (Table 1).

A representative example of the changes of the plasma H-FABP$_c$ level, the plasma CK levels, and the urinary H-FABP$_c$ excretion (Dog 7 in Table 1) is shown in Figure 2. This figure clearly shows that the peak plasma H-FABP$_c$ level occurs much earlier than that of CK, and that peak urinary excretion of H-FABP$_c$ also precedes the peak of plasma CK activity. Urinary excretion of H-FABP$_c$ ceased within 2 h of reperfusion. All these findings were similar in all seven dogs.

In two sham-operated dogs, plasma CK activity showed a slight increase and peaked at the end of the experiment. However, plasma and urinary H-FABP$_c$ was not elevated above the baseline (Table 1).

Estimation of infarct size

The cumulative H-FABP$_c$ and CK release calculated in Dog 7 are shown in Figure 3. H-FABP$_c$ release ceased sharply and reached a plateau within 1 h of reperfusion, while CK release was prolonged and took about 4 h to reach a plateau. Similar results were observed in all seven dogs.

The calculated H-FABP$_c$ release was closely correlated with the infarct size measured by

TABLE 1. H-FABP$_c$ levels in plasma and urine, plasma CK, and infarct size in reperfusion induced myocardial injury

Dog	1	2	3	4	5	6	7	Mean	S.D.	8	9	Mean
Body weight (kg)	12.0	11.0	9.0	9.5	10.0	9.0	8.5	9.9	1.2	10.0	8.0	9.0
Infarct size (g)	6.0	6.2	4.6	3.8	3.1	2.1	4.6	4.3	1.4	0	0	0
Baseline H-FABP$_c$ in plasma (μg/l)	18.1	16.5	5.4	8.7	22.4	18.0	17.9	15.3	5.5	10.1	13.8	12.0
Baseline H-FABP$_c$ in urine (ng/min)	11.8	9.1	26.2	41.6	13.9	23.6	11.0	19.6	10.8	7.3	9.3	8.3
Baseline CK (U/l)	141	258	210	167	677	110	275	263	178	114	247	181
Peak H-FABP$_c$ in plasma (μg/l)	226.6	212.3	138.4	152.9	198.9	173.4	219.4	188.8	31.8	*	14.8	—
Peak H-FABP$_c$ in urine (ng/min)	5038	5835	3751	3365	3990	1684	5710	4196	1359	12	*	—
Peak CK (U/l)	7120	7210	11 800	7390	8250	3540	5250	7223	2375	282	508	395
Time to peak H-FABP$_c$ in plasma (min)	30	30	15	45	20	20	30	27	9	—	45	—
Time to peak H-FABP$_c$ in urine (min)	75	60	75	75	45	45	45	60	14	240	75	158
Time to peak CK in plasma (min)	150	180	105	150	180	270	180	174	47	480	600	540
H-FABP$_c$ release (μg/l)	212.9	238.7	178.2	192.8	182.3	158.1	203.1	195.2	24.2	—	—	—
Total H-FABP$_c$ excretion in urine (μg)	333.6	455.4	228.1	255.6	181.4	74.6	328.5	261.5	114.3	2.4	2.7	2.6
CK release (U/l)	8868	8478	15 292	9799	7983	3662	10 023	9158	3183	—	—	—

Dogs 8 and 9: sham operated dogs. H-FABP$_c$: heart-type cytoplasmic fatty acid-binding protein; CK: creatine kinase; *Below baseline. Time to peak is expressed as a time after reperfusion.

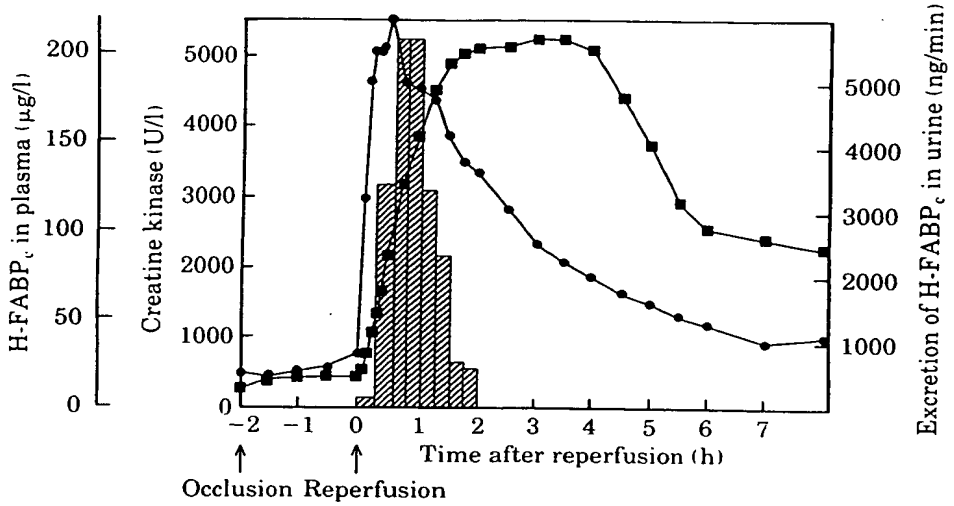

FIGURE 2. Representative time course of the changes of plasma H-FABP$_c$ level (●—●) and urinary excretion of H-FABP$_c$ (bar graph) and plasma CK activity (■—■) after coronary reperfusion. The peak plasma H-FABP$_c$ level and its peak urinary excretion preceded the peak of CK. Excretion of H-FABP$_c$ in urine (bar graph) ceased within 2 h of reperfusion.

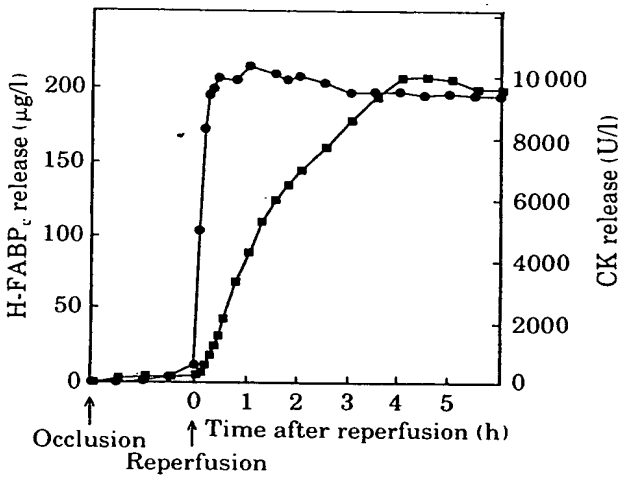

FIGURE 3. Representative cumulative H-FABP$_c$ release (●—●) and CK release (■—■). H-FABP$_c$ release initially increased rapidly and reached a plateau within 1 h of reperfusion, whereas CK release increased gradually and required 4 h to achieve a plateau.

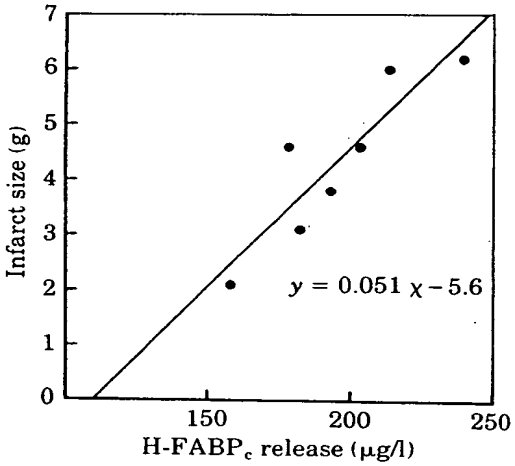

FIGURE 4. Relationship between the calculated H-FABP$_c$ release and infarct size. A significant correlation was observed between H-FABP$_c$ release and infarct size ($r = 0.89$, $r^2 = 0.80$, $P < 0.01$, $n = 7$).

TTC staining ($r = 0.89$, $r^2 = 0.80$, $P < 0.01$, Fig. 4), while no significant correlation was observed between the CK release and infarct size ($r = 0.44$, $r^2 = 0.19$, Fig. 5). A strong correlation was also observed between the total amount of H-FABP$_c$ excreted into the urine and infarct size ($r = 0.94$, $r^2 = 0.88$, $P < 0.01$, Fig. 6).

Kinetic studies of exogenous H-FABP$_c$

A representative example of the elimination of immunoreactive H-FABP$_c$ from the circulation after bolus intravenous injection of exogenous H-FABP$_c$ is shown in Figure 7. H-FABP$_c$ was rapidly eliminated from the circulation. The calculated kinetic parameters are given in Table 2. Figure 8 shows semilogarithmic plots of the plasma H-FABP$_c$ level

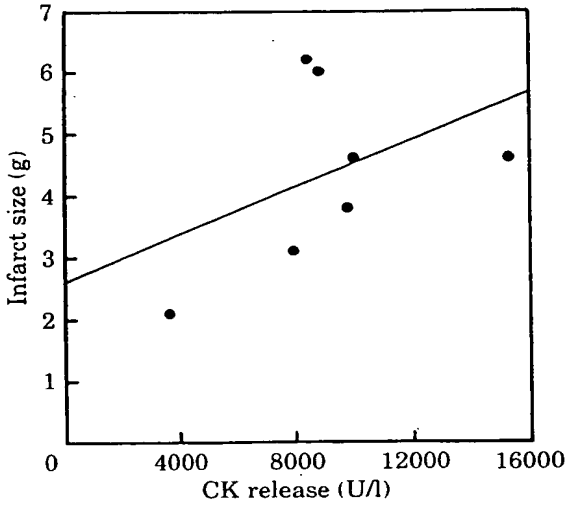

FIGURE 5. Relationship between the calculated CK release and infarct size. No significant correlation was observed between CK release and infarct size ($r=0.44$, $r^2=0.19$, $n=7$).

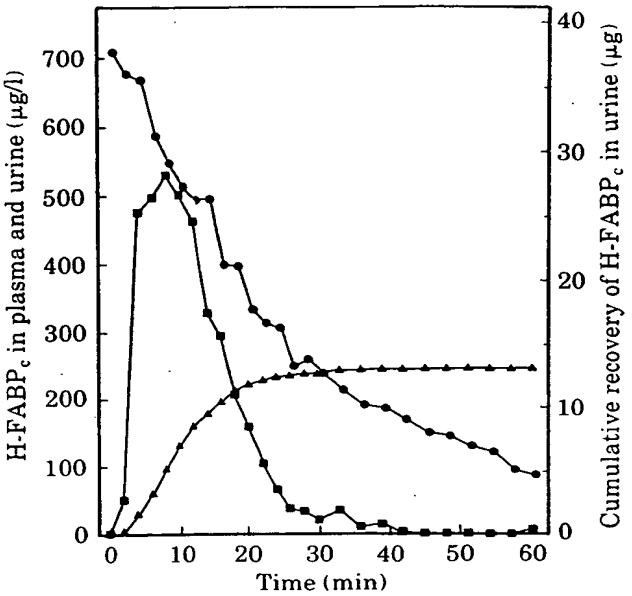

FIGURE 7. Representative elimination and excretion kinetics for H-FABP$_c$ after bolus administration of exogenous H-FABP$_c$. ●—●, plasma H-FABP$_c$ level; ■—■, urinary H-FABP$_c$ level; ▲—▲, cumulative recovery of H-FABP$_c$ in urine.

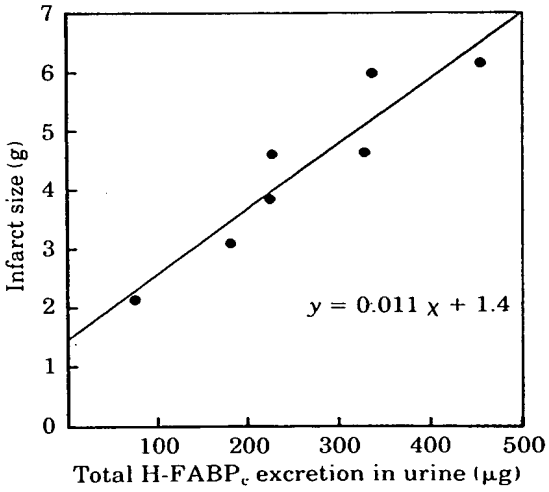

FIGURE 6. Relationship between the total H-FABP$_c$ excretion in urine and infarct size. A close correlation was observed between the total urinary excretion of H-FABP$_c$ and infarct size ($r=0.94$, $r^2=0.88$, $P<0.01$, $n=7$).

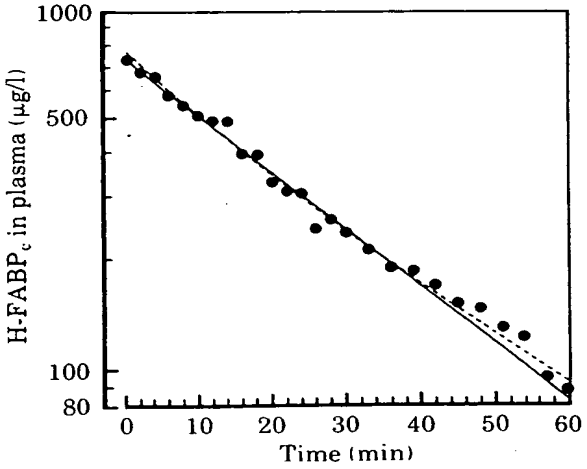

FIGURE 8. Semi-logarithmic plots of plasma H-FABP$_c$ levels *vs* time after bolus administration of H-FABP$_c$. Curves were obtained by fitting the data to a one-compartment (—) or two-compartment model (.....) as described in Materials and Methods.

vs time after exogenous H-FABP$_c$ administration, as well as curves obtained by fitting the data to a one- or two-compartment model with the MULTI program. As can be seen, these two curves closely resembled each other. The elimination rate constant (Ke) calculated with the one-compartment model (0.0275 ± 0.094/min) showed no significant difference compared to that of a two-compartment model (0.0267 ± 0.0092/min). The

TABLE 2. Kinetic parameters for one- and two-compartment model after injection of H-FABP$_c$

	One-compartment					Two-compartment							
Dog	B.W. (kg)	Dose (μg/kg)	Vd (ml/kg)	Ke (min^{-1})	$T_{1/2}$ (min)	$V1$ (ml/kg)	$V2$ (ml/kg)	$K12$ (min^{-1})	$K21$ (min^{-1})	Ke (min^{-1})	$T_{1/2}$ (min)	Yield (μg)	(%)
1	9.5	19.1	66.7	0.0287	24.1	63.3	81.2	0.0136	0.0106	0.0203	34.1	8.8	4.9
2	10.5	24.7	124.1	0.0220	31.5	99.8	28.7	0.0337	0.1170	0.0252	27.5	16.2	6.2
3	14.0	16.6	76.7	0.0216	32.1	70.3	13.1	0.0113	0.0607	0.0213	32.5	14.1	6.0
4	9.0	27.4	129.6	0.0233	29.7	100.4	51.8	0.0327	0.0634	0.0240	28.9	18.3	7.4
5	10.0	20.1	26.7	0.0366	18.9	26.0	3.7	0.0059	0.0413	0.0361	19.2	12.2	6.1
6	8.0	27.1	60.4	0.0172	40.3	60.4	0	0	0.0172	0.0172	40.3	15.0	6.9
7	10.5	18.8	30.4	0.0435	15.9	27.8	9.5	0.0119	0.0350	0.0425	16.3	15.9	8.1
Mean	10.2	22.0	73.5	0.0276	27.5	64.0	26.9	0.0156	0.0493	0.0267	28.4	14.4	6.5
S.D.	1.7	4.0	37.8	0.0087	7.8	27.8	27.6	0.0119	0.0332	0.0085	7.8	2.9	1.0

B.W., body weight; Vd, volume of distribution; Ke, elimination rate constant; $T_{1/2}$, half-time of disappearance; $V1$ and $V2$, volumes of distribution of compartments 1 and 2; $K12$ and $K21$, kinetic rate constants; Yield, urinary recoveries of administrated canine H-FABP$_c$; S.D., standard deviation.

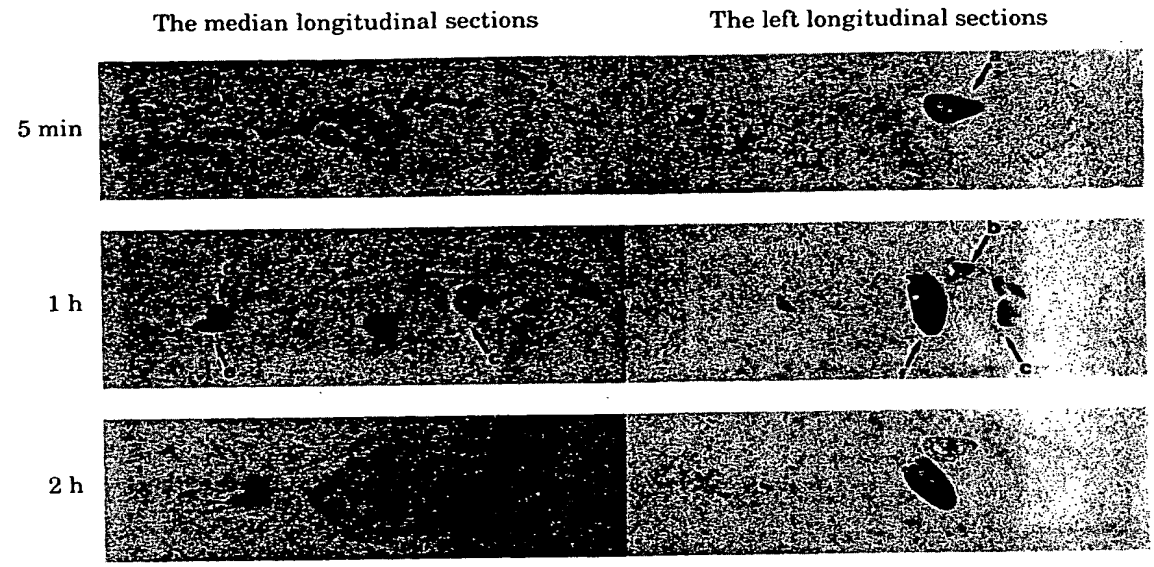

FIGURE 9. Whole-body autoradiographs of mice after the intravenous administration of ^{125}I-H-FABP$_c$ (0.216 μg/kg). a, renal cortex; b, renal medulla; c, bladder; d, thyroid gland; e, salivary glands; f, stomach.

volume of distribution in the one-compartment model ($Vd = 73.5$ ml/kg) did not differ from the sum of the volumes in the two-compartment model ($V1 + V2 = 90.9$ ml/kg, where $V1 > V2$). No difference was observed between AIC value for a one-compartment kinetic model and that for a two-compartment model. Thus, the elimination kinetics of H-FABP$_c$ can be reasonably considered to follow a mono-exponential process (one-compartment model).

Figure 7 also shows the urinary H-FABP$_c$ levels and its cumulative recovery. Consistent with the rapid elimination of H-FABP$_c$ from the circulation, it appeared rapidly in the urine, with the peak at 6.9 ± 2.0 min after administration. The excretion of H-FABP$_c$ into the urine was virtually completed within 60 min and the recovery of the immunoreactivity in urine was 6.5 ± 1.0% of the administered dose within 60 min (Table 2).

Whole-body autoradiography

Figure 9 shows whole-body autoradiographs showing the distribution of radioactive materials after intravenous injection of ^{125}I-H-FABP$_c$ in mice. The radioactivity was highly concentrated in the renal cortex at 5 min after administration. At 60 and 120 min, a high level of radioactivity appeared in the bladder as well as the renal medulla. The thyroid gland, salivary glands, and stomach also contained high levels of the radioactivity. The radioactivity in the salivary glands and stomach disappeared following acid treatment of specimens, which suggested that the radioactivity in these tissues was acid soluble and thus was caused by the release of inorganic radioiodine from ^{125}I-H-FABP$_c$. The radioactivity in the thyroid gland was acid-insoluble and may have been due to macromolecules containing radioiodine as reported previously (Kim *et al.*, 1987; Miyazaki *et al.*, 1988).

To characterize the radioactive materials in the urine, ^{125}I-H-FABP$_c$ was injected intravenously into the dogs, and the urine was collected and concentrated with Centriprep 10 (Amicon, USA). Most of the radioactivity remained in the concentrate, and the filtrate contained only minor radioactivity. The concentrate was then subjected to SDS-PAGE followed by autoradiography, which revealed that most of the radioactivity migrated in a single band corresponding to purified canine H-FABP$_c$. These findings indicate that some part of ^{125}I-H-FABP$_c$ administered intravenously was degraded elsewhere in the body, while undegraded ^{125}I-H-FABP$_c$ accumulated rapidly in the kidneys and was excreted in the urine. The excreted H-FABP$_c$ in the urine was

not degraded as shown in autoradiography of SDS-PAGE, and the immunoreactivity of H-FABP$_c$ in the urine was also preserved.

Discussion

H-FABP$_c$ is a low-molecular weight cytosolic protein that is found predominantly in the heart, with other tissues containing lesser amounts (Crisman *et al.*, 1987; Bass and Manning, 1986). H-FABP$_c$ is immunologically distinguishable from hepatic and small intestinal FBAP$_c$. In addition, a transient increase of the plasma H-FABP$_c$ level has been observed during myocardial injury (Glatz *et al.*, 1988; Knowlton *et al.*, 1989a, 1989b; Kleine *et al.*, 1992; Tanaka *et al.*, 1991). Thus, this protein seems to satisfy the requirements of a biochemical indicator for AMI, as proposed by Puleo and Roberts (1988). Recently, we observed that human H-FABP$_c$ can be detected in plasma and urine shortly after the onset of symptoms in AMI patients and that the urinary H-FABP$_c$ level is higher than the plasma level (Tanaka *et al.*, 1991). However, the precise time course of the plasma and urinary H-FABP$_c$ during myocardial injury and its elimination kinetics were not yet determined.

In this study, we analysed the plasma and urinary H-FABP$_c$ in a coronary occlusion-reperfusion model of AMI. Myocardial infarction was confirmed by TTC staining. The plasma CK activity was used as a reference indicator for myocardial injury, because canine CK-MB activity could not be measured by the commercial CK-MB activity assay kit which is routinely used at our hospital. Production of AMI was attempted in 15 dogs. Three of them died shortly after LAD occlusion and myocardial infarction could not be produced in another five, presumably because of good collateral flow (Schaper *et al.*, 1967). Three of these five dogs showed a slight increase of the plasma H-FABP$_c$ level when compared with before coronary ligation as well as minor or transient ECG changes, while the other two showed no increase of the plasma H-FABP$_c$ level and no ECG changes. In all these five dogs, the urinary H-FABP$_c$ was not elevated and the CK activity changes were similar those from controls. We have no appropriate method of confirming the degree of ischaemia or very slight myocardial injury. Since the aim of this study was to clarify the appearance of H-FABP$_c$ in the circulation and urine during myocardial infarction as well as to examine the relationship between H-FABP$_c$ and infarct size, the data from these five dogs without definite myocardial infarction were not used.

In the dogs with confirmed myocardial infarction, H-FABP$_c$ appeared in the circulation just after reperfusion, as has been reported in the rat by Knowlton *et al.* (1989b). The peak plasma H-FABP$_c$ level occurred before that of CK. Cumulative H-FABP$_c$ release showed a plateau about 1 h after reperfusion, while cumulative CK release required about 4 h to reach a plateau. These findings indicate that H-FABP$_c$ leaked more readily and more rapidly from injured myocardial cells than CK. This difference in leakage would be expected from the characteristics of H-FABP$_c$ (i.e., an M_r of 14–16 kDa and an cytosolic localization (Crisman *et al.*, 1987; Brorchers *et al.*, 1989) and CK (i.e., an M_r of 81 kDa and partial binding to structural elements (Ottaway, 1967).

In order to clinically apply H-FABP$_c$ as an indicator of myocardial injury, it is necessary to know its elimination kinetics. Therefore, the disposition of H-FABP$_c$ was investigated in dogs given an exogenous H-FABP$_c$. The kinetic study showed that H-FABP$_c$ underwent mono-exponential elimination from the circulation (one-compartment model). Its elimination rate constant (Ke) was 0.0275 ± 0.0094/min and its disappearance half-time ($t_{1/2}$) was 27.5 ± 8.4 min. Due to the rapid clearance of myoglobin from the circulation ($Ke = 0.132$/min and $t_{1/2} = 5.5$ min (Ellis and Saran, 1989), its levels have been used as a non-invasive indicator of successful recanalization therapy and/or reinfarction (Kagen *et al.*, 1977; Ellis *et al.*, 1988). H-FABP$_c$ is also rapidly and constantly cleared from the circulation, so serial measurements of this protein may be used to detect reinfarction, as suggested by Kleine *et al.* (1992). In addition, we have previously reported the usefulness of serial measurement of plasma H-FABP$_c$ during successful recanalization therapy (Abe *et al.*, 1991).

Infarct size is a major determinant of the prognosis of AMI, so a good assessment of infarct size is essential (Braunwald, 1989; Simoon *et al.*, 1986; Van de Werf and Arnold, 1988). The size of infarcts has been evaluated clinically from the peak CK activity and the myosin light chain concentration (Isobe *et al.*, 1989), as well as by analysis of CK-time activity curves (CK release) (Shell *et al.*, 1971; Norris *et al.*, 1975). In this study, H-$FABP_c$ release was calculated using the method of Shell *et al.* (1971) and it showed a good correlation with infarct size, while a poor correlation between CK release and infarct size was observed. This difference might have been attributed to incomplete leakage of CK from the injured myocardium during the study period, CK leakage from skeletal muscle or small number of observations. However, even in the relatively short observation period and in small number of observations in this study, H-$FABP_c$ release showed a good correlation with infarct size.

Urinary H-$FABP_c$ levels became elevated soon after reperfusion, and the total amount of H-$FABP_c$ in the urine showed a close correlation with infarct size. But some problems may arise when applying an urinary H-$FABP_c$ for the estimation of infarct size. The major problem seems to be the relatively low recovery of H-$FABP_c$ in urine. In the kinetics study, the urinary recovery of immunoreactive H-$FABP_c$ was $6.5 \pm 1.0\%$, a similar proportion to that for myoglobin (Klocke *et al.*, 1982). The excretion of H-$FABP_c$ into the urine was examined using ^{125}I labelled H-$FABP_c$. Some part of radioactivity bound to H-$FABP_c$ rapidly appeared in the urine and the urinary radioactive material was mainly composed of undegraded H-$FABP_c$. The renal handling of low molecular weight proteins is affected by many factors, such as renal blood flow, perfusion pressure, glomerular filtration, and tubular reabsorption. Moreover, the metabolism of H-$FABP_c$ has not yet been studied and is still unclear, so further investigations are necessary to elucidate its metabolism and excretion under various conditions.

If the normal H-$FABP_c$ level in plasma and urine was high or showed wide range, precise assessment of myocardial injury would be difficult. In this study, a relatively high H-$FABP_c$ level was detected even before myocardial injury was produced. The baseline H-$FABP_c$ level was $15.3 \pm 5.5\ \mu g/l$ in plasma and $19.4 \pm 6.2\ \mu g/l$ in urine. The baseline plasma H-$FABP_c$ level is of similar to that reported by Knowlton *et al.* (1989b) in the rat. However, the normal plasma human H-$FABP_c$ level is reported to be much lower than that in animals (0–$2.8\ \mu g/l$ by Tanaka *et al.* (1991) and $1.9\ \mu g/l$ by Kleine *et al.* (1992). The precise reason for this difference between humans and animals is obscure. $FABP_c$s from heart and skeletal muscle are identical (Peeters *et al.*, 1991). Polyclonal anti-canine H-$FABP_c$ antibodies used in this study also reacted to skeletal muscle $FABP_c$. So experiment conditions such as anaesthesia, thoracotomy and/or mongrel dogs used in this study may influence the high baseline plasma and urinary H-$FABP_c$ levels observed in this study. High baseline CK activity in this study may also due to the same reasons. However, the mean $FABP_c$ content of skeletal muscle is reported to be relatively low (Peeters *et al.*, 1991), and H-$FABP_c$ in normal human plasma and urine is also reportedly low with a narrow range (Kleine *et al.*, 1992; Tanaka *et al.*, 1991). Therefore, elevation of H-$FABP_c$ might still be a sensitive indicator of myocardial injury.

The remaining problem is the convenience and rapidity of H-$FABP_c$ measurement, but this can be overcome by technical improvement. In this study and a previous study (Tanaka *et al.*, 1991), we used a C-EIA with polyclonal anti-H-$FABP_c$ antibodies, which was rather time-consuming. We are now developing a human H-$FABP_c$ assay kit using monoclonal anti-human H-$FABP_c$, which should allow many samples to be analysed simultaneously in about 1 h. Thus, much more information might soon be collected about the H-$FABP_c$ level under normal and pathological conditions, including myocardial injury, transient or prolonged myocardial ischemia, and skeletal muscle disorders.

In summary, the plasma and urinary H-$FABP_c$ reflected the occurrence of myocardial injury produced by coronary occlusion–reperfusion in dogs as well as its severity. Thus, plasma and urinary H-$FABP_c$ might be useful as early indicators of myocardial injury and for the estimation of infarct size.

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Serum Amyloid A Remains at Physiological Concentrations in Coronary Atherosclerosis

To the Editor:

Serum amyloid A (SAA), an apolipoprotein found in HDL, is a sensitive acute-phase reactant, its concentration in serum increasing up to 1000-fold in inflammatory disorders. Recently, greater attention has been paid to the roles of SAA in lipoprotein metabolism and atherogenesis under inflammatory conditions. Findings have shown that (*a*) SAA maintains the reverse cholesterol transport system *[1]*, (*b*) cells in the artery walls are able to express SAA *[2]*, and (*c*) SAA is present in atherosclerotic lesions *[3]*. However, there are no data regarding serum concentrations of SAA in the atherosclerotic diseases. Because the development of atherosclerotic lesions is accompanied by inflammation-like events, including release of cytokines capable of inducing SAA synthesis from cells in the artery walls *[4]*, a slight increase in serum concentrations of SAA seems possible.

Currently, a well-established latex agglutination nephelometric immunoassay is used to determine SAA *[5]*. The antibodies used in the assay system have no apparent cross-reactivity with a recently discovered constitutive isotype of SAA. This method has shown SAA to be a useful marker for viral infections, in which acute-phase reactants are not remarkably increased *[6]*. Here, we report our examination of serum SAA concentrations in patients with coronary atherosclerosis.

We studied 24 patients (17 men, 7 women; ages 39–90) believed, on the basis of symptoms, stress electrocardiogram, and echocardiogram, to have angina pectoris or myocardial infarction. Stenosis in branches of the coronary artery was finally confirmed by angiography. These patients were followed-up conservatively as outpatients; serum samples were obtained during their regular appointments, when no apparent symptoms were noted.

The SAA values of the patients ranged from 1.5 to 5.1 mg/L. The number of affected coronary artery branches did not affect the SAA concentrations. The concentrations of HDL cholesterol were reduced (mean 30% lower) in these patients, but the SAA values were not related to the HDL cholesterol concentrations.

Using this method in a previous study *[7]*, we had also measured SAA in a large number of healthy subjects (n = 452, ages 16–70 years). In 95% of these healthy subjects, SAA values were <8.0 mg/L, and 85% showed concentrations <5.0 mg/L. Thus, the SAA concentrations in coronary atherosclerosis are indistinguishable from those seen physiologically. This suggests that local atherosclerotic changes as inflammatory stimuli may not reach the liver, the central organ of SAA synthesis; alternatively, perhaps locally produced SAA does not appear in the circulation.

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Increased Fatty Acid-Binding Protein Concentration in Plasma of Patients with Chronic Renal Failure

To the Editor:

The soluble cytoplasm of most cells contains low-molecular-mass (14–15 kDa) proteins able to bind long-chain unesterified fatty acids. Of these so-called fatty acid-binding proteins (FABP), nine different types have been identified *[1, 2]*. Heart and skeletal muscles contain the same type of FABP [referred to as heart-type (H)-FABP] *[1, 2]*, but its concentration in the heart is severalfold higher than that in the skeletal muscles *[3]*. The concentration of FABP in the plasma of healthy persons is relatively low (2–6 $\mu g \cdot L^{-1}$) *[4]*. FABP is released from the heart early after the onset of infarction, whereafter its plasma concentration increases manyfold *[3–6]*. Increased excretion of FABP in urine also occurs after infarction *[5, 7]*. Several recent studies indicate the usefulness of the plasma FABP concentration as an early biochemical marker for myocardial infarction diagnosis *[3, 5, 7]*. However, to interpret properly the values of plasma

FABP concentration, one has to take into account not only its source and rate of release into plasma but also its elimination from plasma. It is obvious that any change in the clearance rate of FABP would affect its plasma concentration, and thus may lead to erroneous interpretation. Kleine et al. *[8]* reported a patient with acute myocardial infarction and severe renal insufficiency in whom the plasma FABP concentration remained increased for the whole course of blood sampling (25 h after the infarction), whereas in patients with normal kidney function it normalized in ~10 h after the infarction. Unfortunately, preinfarction data on plasma FABP in this patient were not available. Low-molecular-mass proteins such as FABP and myoglobin are cleared mostly by the kidney *[9, 10]*. As it remains an open question whether, and, if so, to what extent an insufficiency of the kidneys affects the plasma FABP concentration in patients with heart and skeletal muscles intact, we studied plasma FABP and myoglobin in patients with chronic renal failure.

Blood samples were obtained from 15 blood donors (males) and 27 chronically hemodialyzed patients with renal failure (18 males, 9 females, ages 17–66 years; period of dialysis 2–70 months). Their primary renal diseases were: chronic glomerulonephritis (n = 14), interstitial nephritis (n = 2), acute renal failure (n = 3), adult dominant polycystic kidney disease (n = 3), hypertensive nephropathy (n = 3), diabetes mellitus (n = 1), and amyloidosis (n = 1). The patients were clinically stable and free of any severe intercurrent illnesses. They had no clinical evidence of severe secondary hyperparathyroidism. Hemodialysis was performed three times a week with the double needle technique, with cuprophane capillary dialyzers, and with bicarbonate as buffer in the dialysate. The membrane allows the passage of low-molecular-mass solutes up to ~2 kDa. Vascular access was in all cases a Cimino-Brescia arteriovenous fistula. Blood samples were obtained immediately before and after dialysis.

Plasma FABP concentration was measured by a sensitive noncompetitive sandwich ELISA *[4]*. Plasma concentration of myoglobin was measured with a turbidimetric immunoassay (Unimate 3 MYO; Roche Diagnostic Systems, Basel, Switzerland) on a Cobas Mira Plus analyzer (Roche). The concentrations of urea and creatinine in plasma were measured by the urease method and Jaffe reaction, respectively.

The significance of the differences between the means was evaluated statistically by unpaired and paired Student *t*-tests, where appropriate. Correlations between plasma FABP and (or) myoglobin concentrations and the period of dialysis, and plasma urea and creatinine concentrations were determined by Pearson product–moment correlation, and the level of significance was taken at $P < 0.05$.

Plasma creatinine and urea concentrations were high before dialysis and dropped markedly after dialysis (Table 1). The mean plasma concentration of FABP in the uremic patients before and after dialysis was 21 and 25 times higher, respectively, than that in the blood donors. The mean plasma myoglobin concentration in the uremic patients before and after dialysis was 3.7 and 4.0 times higher, respectively, than that in the blood donors. The insignificant increase in plasma concentrations of FABP and myoglobin after dialysis may reflect removal of blood water during dialysis. In the patients, before dialysis the mean myoglobin/FABP ratio was five times lower than in the donors, and after dialysis six times lower (Table 1). Neither plasma FABP nor plasma myoglobin concentrations showed a correlation with the period of dialysis or urea or creatinine concentration in plasma.

The present data are the first to show that plasma FABP concentration is markedly increased in patients with chronic renal failure and normal heart function, similar to that found for myoglobin *[11]*. It is clear that a certain amount of each protein must be constantly removed either by the kidney or by other tissues, thus preventing progressive increase in the concentration with time of renal failure. Interestingly, the plasma FABP concentration is much higher (20–25 fold) than that of myoglobin (fourfold) despite the fact that these proteins have similar molecular masses (15 and 18 kDa, respectively) and show a similar plasma release curve in patients with acute myocardial infarction and normal renal function *[3]*. These findings suggest that the kidneys play a more dominant role in the clearance of plasma FABP than of myoglobin.

The ratio of the concentrations of myoglobin over that of FABP is lower in the heart (ratio ~5) than in skeletal muscles (20–70, depending on muscle type) *[3]*. The use of the ratio of the plasma concentrations of myoglobin over that of FABP to discriminate between heart and skeletal muscle tissue injury has been sug-

Table 1. Plasma FABP and myoglobin concentrations in controls and patients with chronic renal failure.

Subjects		Creatinine, mg %	Urea, mg %	Myoglobin $\mu g \cdot L^{-1}$ (range)	FABP, $\mu g \cdot L^{-1}$ (range)	Myoglobin/FABP (ratio)
Control (n = 15)		0.77 ± 0.14	25.1 ± 7.7	45.8 ± 20.7 (22.3–96.8)	3.0 ± 1.4 (1.4–5.0)	16.2 ± 4.1 (10.9–25.12)
Renal failure patients (n = 27)	B	11.4 ± 3.3	118.0 ± 30.2	170.6 ± 61.8 (63.8–290.1)	62.8 ± 25.2 (12.1–118.2)	3.2 ± 1.8 (0.7–9.6)
	A	4.6 ± 1.3	53.9 ± 18.6	181.3 ± 67.6 (70.2–297.6)	75.5 ± 28.9 (13.6–120.9)	2.7 ± 1.2 (1.2–6.7)

B, before dialysis; A, after dialysis.

gested [3]. Because of the relatively longer increase in plasma FABP compared with myoglobin, the ratio calculated for uremic patients (~3) is similar to that found in patients after heart infarction. Thus, with respect to the discrimination of myocardial from skeletal muscle injury, the decrease of the ratio in chronic renal failure indicates the limitation of the use of this ratio for this purpose.

Serial monitoring of the plasma FABP concentration can also be used to estimate infarct size [6]. However, our results indicate that if the myocardial infarction occurred in a patient with chronic renal failure, the plasma FABP concentration would be relatively higher than in a patient with intact kidneys, thus leading to overestimation of infarct size. Since preinfarct values differ widely among patients, a judgment about infarct size cannot be made.

In conclusion, our data indicate that in patients with chronic renal failure the plasma concentrations of the biochemical markers FABP and myoglobin each are markedly increased. Thus, caution must be taken when using these marker proteins for early diagnosis of myocardial infarction, in case of renal insufficiency, as the preinfarct plasma concentration is very likely to be already high.

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Automated Immunoassay of Cardiac Troponin I in Serum Evaluated

To the Editor:

We evaluated the Opus Troponin I assay (Behring Diagnostics, Westwood, MA), a two-site sandwich, fluorogenic ELISA that uses two goat polyclonal antibodies directed against different protein segments unique to cardiac troponin I (cTnI) [1]. Pipetting, incubations, measurements, and data-reduction steps are performed on the Opus analyzer; the first test result requires 20 min. The assay measures concentrations of cTnI in serum as great as ~135 μg/L.

The calibration appeared to be stable for at least 4 weeks. Serial dilution of a human serum sample with a high concentration of cTnI showed no significant curvature when the curve obtained was tested for linearity [quadratic regression: $y = -0.25 + 96.1x + 6.31x^2$, with the coefficient of x^2 not significantly different from 0 ($P = 0.27$)] [2]. Linear regression analysis of these data confirmed the high linearity of the response ($r = 0.9998$). The minimum detectable cTnI concentration, assessed by 10 replicate measurements of a human serum containing no detectable cTnI concentration and defined as the cTnI value corresponding to the fluorescence signal 3 SD greater than the mean found for this serum, was estimated as 0.38 μg/L. The Opus analyzer, however, reports results <0.50 μg/L as "<0.5 μg/L."

Assay reproducibility was tested by assaying in duplicate, once a day for 10 days, two serum samples with concentrations distributed over the measuring range and the three kit controls containing human cTnI [3]. Analysis of variance showed within-run CVs between 3.4% and 7.2% and total CVs between 5.6% and 13.0%. No interferences were detected in assays of lipemic (triglycerides <10 g/L) or hemolyzed (hemoglobin <2.5 g/L) specimens; concentrations of bilirubin >50 mg/L spuriously increased the reported cTnI concentrations in serum.

To compare the Opus assay with the cTnI Pasteur immunoenzymometric assay (Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France; performed manually according to the manufacturer's current protocol [4]), we assayed 85 unselected individual serum samples with detectable cTnI concentration (>0.04 μg/L as measured with the Pasteur assay). The correlation was good ($r = 0.959$), but the data showed considerable scatter ($S_{y|x} = 10.6$ μg/L), the Opus results being relatively higher within the mid-range of values but equal to

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Influence of renal function on serum and urinary heart fatty acid-binding protein levels

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Background. **Serum heart fatty acid-binding protein (H-FABP) has been reported to be a sensitive and early indicator of myocardial damage. However, circulating H-FABP may be cleared considerably from kidney, similar to that found for myoglobin. Therefore, the possibility exists that any change in renal function affects serum H-FABP concentration, and thus leads to erroneous interpretation. To evaluate the influence of renal function on H-FABP levels, we conducted a prospective study.**

Methods. **Nineteen patients undergoing isolated primary coronary artery bypass grafting were enrolled in this study. The patients were classified by the preoperative creatinine clearance into two groups: the control group (n=12); patients with creatinine clearance of 40 mL/min or greater, and the renal dysfunction group (n=7); patients with creatinine clearance of less than 40 mL/min. Serum H-FABP, CK-MB, troponin-T and urinary H-FABP levels were measured perioperatively.**

Results. **None of the patients had perioperative myocardial infarction. No significant differences were found in CK-MB and troponin-T levels between the groups. The renal dysfunction group resulted in significantly ($p<0.05$) higher serum H-FABP levels and lower urinary H-FABP levels than those in the control group, postoperatively. The creatinine clearance correlated inversely with the peak levels of serum H-FABP ($r=-0.75$, $p=0.0001$) and correlated with the peak levels of urinary H-FABP ($r=0.64$, $p=0.003$).**

Conclusions. **The results indicate that the kidneys play an important role in the clearance of serum H-FABP. Thus, caution must be taken in interpreting this marker for myocardial damage during cardiac surgery in patients with renal dysfunction.**

KEY WORDS: Carrier proteins - Fatty acids - Myocardial infarction - Coronary artery bypass - Kidney physiology.

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The accurate estimation of the extent of myocardial damage during cardiac operation is essential for the evaluation of cardioprotective techniques and for the decision of perioperative patient management. Currently, a number of biochemical markers, such as MB isoenzyme of creatine kinase, troponin and myosin chains, are used clinically and the usefulness of their measurement for the evaluation of myocardial tissue damage has been reported.[1-3] Recently, heart fatty acid-binding protein (H-FABP) has been introduced as a cardiospecific marker for the early assessment of myocardial tissue damage in patients with myocardial infarction and in patients undergoing cardiac surgery.[4-7] In cardiac operations, serum H-FABP level was reported to peak within one hour after reperfusion and the level correlated with aortic cross-clamp time or postoperative catecholamine requirements.[7] It is also reported that the protein appears rapidly in urine and that the urinary level correlates with the severity of the myocardial injury.[8] Thus, serum and urinary H-FABP are being accepted as an early and sensitive biochemical marker for the diagnosis of myocardial injury. However, because low-molecular-mass proteins, such as myoglobin and H-FABP, are considered to be cleared mainly from kidney,[8,9] the possibility exists that any change in the clearance rate of H-FABP from kidney affects its serum concentration, and thus leads to erroneous interpretation.

To date, however, it is unknown whether renal function affects the serum and urinary levels of H-FABP in cardiac surgical patients. Therefore, the

TABLE I.—*Clinical and operative data.*

Parameters	Control (n=12)	Renal dysfunction (n=7)	p values
Age (yrs)	64.2±3.2	66.6±4.8	0.67
Sex (male/female)	9/3	4/3	0.62
Body surface area (m^2)	1.56±0.06	0.52±0.07	0.72
LVMI (g/m^2)	112±7	119±4	0.43
Preoperative Ccr (mL/min)	55.4±3.8	18.6±5.6	0.0001
CHF (Y/N)	0/12	0/7	1.0
Previous myocardial infarction (Y/N)	6/6	3/4	0.99
Diseased vessels (2/3)	1/11	1/6	0.99
LVEF (40-60/>60%)	2/10	1/6	0.99
CPB time (min)	120±3	128±16	0.67
XCL time (min)	86±6	83±18	0.76
No. of distal anastomoses	2.9±0.1	2.8±0.3	0.69
No. of arterial grafts	1.2±0.2	1.2±0.1	0.93

Where applicable, data are shown as the mean ± the standard error of the mean. The p values refer to the results of unpaired Student's "t"-test for continuous variables and χ^2 test or Fisher's exact test for categoric variables. Ccr: creatinine clearance; CHF: congestive heart failure; Control: control group; CPB: cardiopulmonary bypass; LVEF: left ventricular ejection fraction; LVMI: left ventricular mass index; Renal dysfunction: renal dysfunction group; XCL: aortic cross-clamp.

present study was undertaken to evaluate the influence of renal function on the perioperative serum and urinary H-FABP levels in patients undergoing coronary artery bypass grafting.

Materials and methods

Patient population

Nineteen patients undergoing primary isolated coronary artery bypass grafting between March 1999 and October 1999 were enrolled in this study. All patients signed a consent form approved by the Human Experimental Committee of Kurume University. Exclusion criteria for the study were prior myocardial infarction within 30 days before surgery, unstable angina pectoris, left ventricular hypertrophy indicated by left ventricular mass index of greater than 150 g/m^2, hepatic disease as indicated by aspartate transamynase, alanine aminotransferase or bilirubin levels of more than 1.5 times the upper limit of normal, and peripheral vascular disease. The patients were classified by the preoperative renal function into two groups: the control group (n=12); patients with creatinine clearance (Ccr) of 40 mL/min or greater, and the renal dysfunction group (n=7); patients with Ccr of less than 40 mL/min. The Ccr levels were measured in triplicate for all patients and the mean values were used for the classification. The demographic data are shown in Table I.

Technique of operation

Induction and maintenance of anesthesia were standardized.[10] The components of the extracorporeal perfusion circuit consisted of a capillary membrane oxygenator, a hard-shell cardiotomy reservoir, an arterial filter (CAPIOX SX (HP), Terumo Corp. Tokyo, Japan), an arterial cannula, a two-staged venous cannula, and a cardiotomy suction (Duraflo II, Baxter Healthcare Corp, Irvine, CA). Before CPB was initiated, heparin sodium was administered at an initial dose of 300 IU/kg. Additional heparin was administered if the celite-activated clotting time (Hemochron 401, International Technidyne Corp, Edison, N.J.) became less than 500 seconds. During CPB, the hematocrit was maintained between 20% and 25%, the perfusion flows were kept between 2.4 and 2.5 $L \cdot min^{-1} \cdot m^{-2}$, and mean arterial pressure was maintained between 50 and 60 mmHg. Systemic temperature was maintained at 30±1°C. During aortic cross-clamping, the myocardium was protected with intermittent antegrade cold blood cardioplegia given at approximately 15 min intervals. Distal and proximal anastomoses were constructed in an alternating manner during a single period of aortic cross-clamping. Rewarming of all patients was commenced during construction of the last anastomosis. A left internal mammary artery graft was anastomosed to the left anterior descending coronary artery as the last graft in all patients. CPB was terminated at a rectal temperature of 36°C. Heparin was neutralized by continuous intravenous admin-

istration of protamine sulfate over a 5-min period. Postoperative intensive care unit (ICU) care followed the standard protocol of this institute.

Measurement of sample

Blood and urine samples were collected immediately after induction of anesthesia, 30 minutes, 1, 2, 3, 6 and 18 hours after aortic declamping. All blood samples for determination of H-FABP, CK-MB and troponin-T were drawn in precooled vacuum tubes and were immediately centrifuged (1500 g for 10 minutes) at -4°C. The serum was transferred to a sterile polypropylene test tube and stored at -80°C until assayed. Urine samples were collected in a sterile polypropylene test tube and stored at 80°C, until assayed. The urine volume collected for 5 minutes was measured (BARD Urotrack 224, C.R. Bard Inc., Covington, GA) and presented as urine volume per minute. Because urinary H-FABP levels varied over a wide range, depending on time-to-time urinary flow variation, urinary H-FABP was expressed as excretion rate; H-FABP excretion rate (ng/min) = urinary H-FABP level (ng/mL) × urine volume per minute (mL/min).[9] The H-FABP levels in serum and urine were determined by a sandwich enzyme immunoassay with two different anti-H-FABP monoclonal antibodies (Markit-M H-FABP, DainipponPharmceutical Co., Ltd, Osaka, Japan) and the reference range for serum level was less than 6.2 ng/mL. Activity of CK-MB was measured with immunoinhibition of the predominant M unit in creatine kinase (MERCKauto CK-MB kit, Kanto Chemical Co., Inc., Tokyo, Japan) and the reference range was 6 to 28 IU/L. Troponin-T levels were measured with an enzyme immunoassay (Immunoassay-Test, Boehringer Mannheim, Germany) and the reference range was less than 0.25 ng/mL. No adjustment was made for hemodilution.

Diagnosis of perioperative myocardial infarction

Perioperative myocardial infarction was diagnosed when patients showed elevation of more than 1 mm of the ST-segment, T-wave inversion or new persisted Q waves in the postoperative electrocardiograms obtained immediately after and at 3, 12 and 24 hours after admission of the patients to ICU with either a concomitant increase of CK-MB level of greater than 50 IU/L or troponin-T level of greater than 3.5 ng/mL.

Statistical analysis

Statistical analysis was performed with StatView 5.0 software (SAS Institute Inc., Cary, NC). All data are expressed as a mean ± standard error of the mean. One-way or two-way repeated measures analysis of variance (ANOVA) was used to test the group and time on the levels of serum H-FABP, CK-MB, troponin-T and urinary H-FABP. When analysis of variance indicated a significant effect of the group or time ($p<0.05$), the differences were specified with Sheffe's test for within-group comparison and unpaired Student's "t"-test for between-groups comparison. The Pearson's correlation coefficient test was used to explore the correlation between the levels of biochemical markers and renal function. Unpaired Student's "t"-test was used to compare other continuous variables. Categoric data were analyzed using the χ^2 test or Fisher's exact test where appropriate. Statistical significance was assumed at a probability level of less than 0.05.

Results

Clinical and operative data for the two patient groups are shown in Table I. No significant differences except for preoperative renal function were noted in the clinical and operative data.

Clinical outcome

None of the 19 patients in this series died. None of the patients had perioperative myocardial infarction and a low-output syndrome postoperatively (defined as a requirement for inotropic medication or balloon pump support, or both, because of a cardiac index of less than 2.1 $L \cdot min^{-1} \cdot m^{-2}$ and systolic blood pressure of less than 80 mmHg, despite an adequate preload and correction of any electrolyte disorders).

Time course of serum and urinary biochemical markers

Time course of serum H-FABP, CK-MB, troponin-T levels and urinary H-FABP excretion rate in two groups are shown in Figure 1. Preoperative CK-MB, troponin-T levels and urinary H-FABP excretion rate did not differ between the groups, whereas serum H-FABP level was significantly higher in the renal dysfunction group than that in the control group. No significant differences were found in CK-MB (group

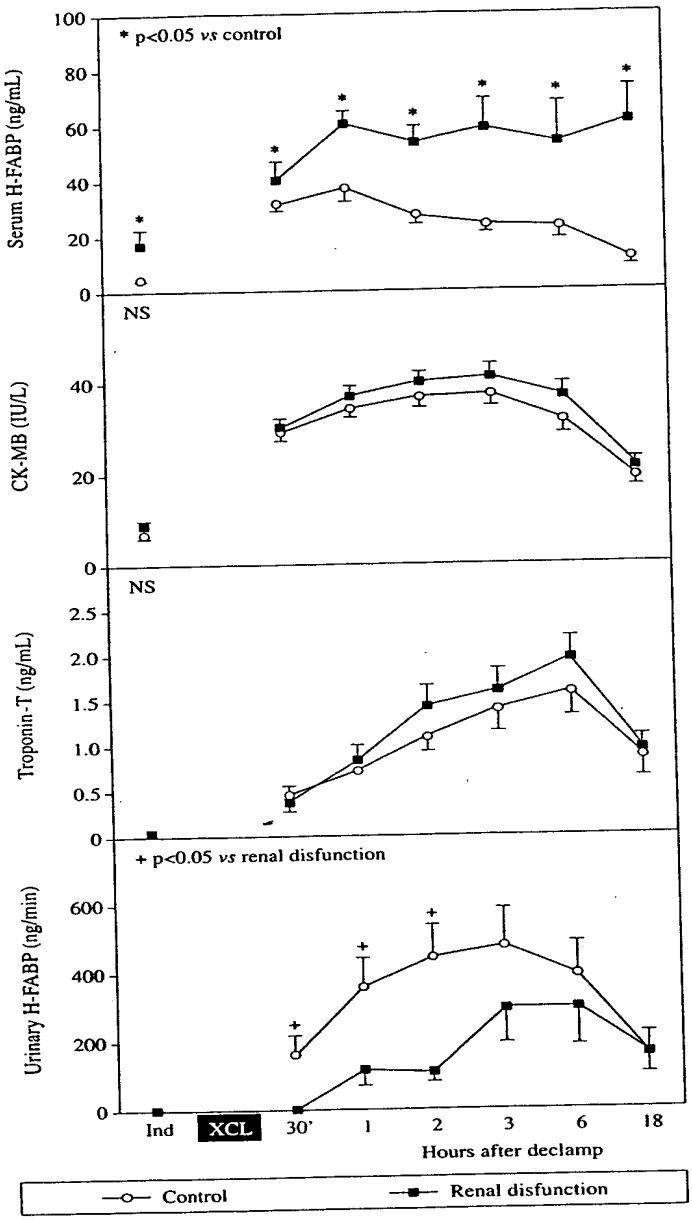

Fig. 1.—Time course of serum H-FABP, CK-MB, troponin-T levels and urinary H-FABP excretion rate. No significant differences were found in CK-MB and troponin-T levels between the groups at any time. Serum H-FABP levels were significantly higher immediately after induction of anesthesia, 1, 2, 3, 6 and 18 hours after aortic declamping in the renal dysfunction group than those in the control group. Urinary H-FABP excretion rate was significantly lower in the renal dysfunction group 30 minutes, 1 and 2 hours after aortic declamping. Control: control group; Ind: induction of anesthesia; Renal dysfunction: renal dysfunction group; XCL: crossclamp.

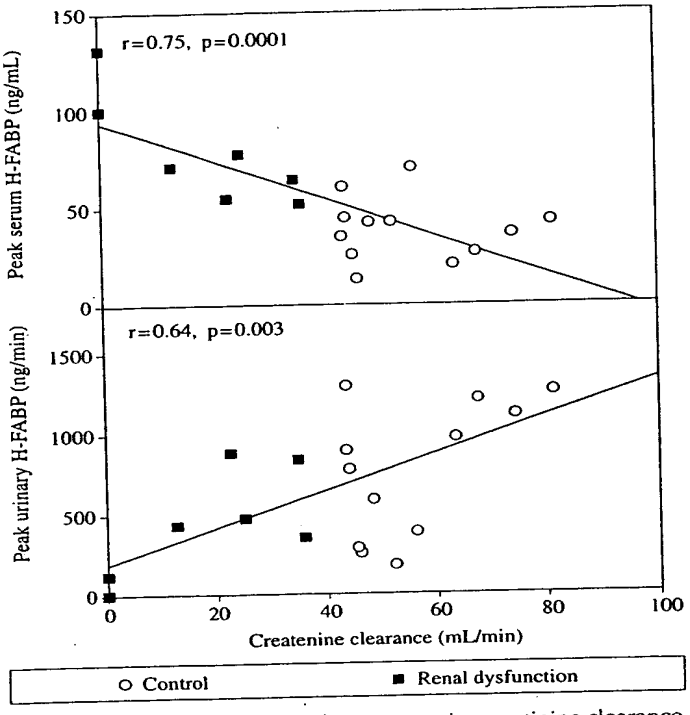

Fig. 2.—Relationship between the preoperative creatinine clearance and the peak serum H-FABP levels, and between the preoperative creatinine clearance and the peak urinary H-FABP excretion rate. The preoperative Ccr levels correlated inversely with the peak serum H-FABP levels inversely (r=-0.75, p=0.0001) and correlated positively with the peak urinary H-FABP excretion rate (r=0.641, p=0.003). Ccr: creatinine clearance; Control: control group; Renal dysfunction: renal dysfunction group.

effect, p=0.12; time effect, p=0.001 by two-way repeated measures ANOVA) and troponin-T (group effect, p=0.11; time effect, p=0.0001 by two-way repeated measures ANOVA) levels after aortic declamping between the groups. Serum H-FABP levels reached peak values one hour after aortic declamping in the control group, whereas the increase persisted until 18 hours after aortic declamping in the renal dysfunction group (group effect, p=0.001; time effect, p=0.0001 by two-way repeated measures ANOVA). The levels were significantly higher 1, 2, 3, 6 and 18 hours after aortic declamping in the renal dysfunction group than those in the control group (p<0.05 by Student's "t"-test). Urinary H-FABP excretion rate was significantly lower in the renal dysfunction group 30 minutes, 1 and 2 hours after aortic declamping (group effect, p=0.03; time effect, p=0.0001 by two-

way repeated measures ANOVA; $p<0.05$ by Student's "t"-test). There was a positive correlation between the peak serum H-FABP levels and the peak CK-MB levels ($r=0.72$, $p=0.007$) and between the peak serum H-FABP levels and the peak troponin-T levels ($r=0.75$, $p=0.004$) in the control group, whereas no significant correlation was observed between the peak serum H-FABP levels and the peak CK-MB levels ($r=-0.30$, $p=0.54$) and between the peak serum H-FABP levels and the peak troponin-T levels ($r=-0.35$, $p=0.47$) in the renal dysfunction group.

Relationship between the creatinine clearance and biochemical markers

The relationship between the preoperative creatinine clearance and the peak serum H-FABP levels and between the preoperative creatinine clearance and the peak urinary H-FABP excretion rate is shown in Figure 2. Although no significant correlation was observed between the preoperative creatinine clearance and the peak CK-MB levels ($r=-0.04$, $p=0.88$), or between the preoperative creatinine clearance and the peak troponin-T levels ($r=-0.44$, $p=0.07$), the preoperative creatinine clearance levels correlated inversely with the peak serum H-FABP levels ($r=-0.75$, $p=0.0001$) and correlated positively with the peak urinary H-FABP excretion rate ($r=0.641$, $p=0.003$).

Discussion and conclusions

H-FABP is a low-molecular weight cytoplasmic protein (14-16 kDa) that is abundant in cardiomyocytes and is thought to be involved in the uptake, transport and metabolism of fatty acid.[11] As this protein features a low-molecular weight and a cytosolic localization, it easily leaks out from the injured myocardium and rapidly appears in circulating blood and urine.[9 11] Recent reports[4-7] have suggested the possibility of this protein as a biochemical marker for the early assessment of myocardial tissue damage in patients with myocardial infarction and in patients undergoing cardiac surgery. In a dog model of ischemia and reperfusion, H-FABP levels in serum and urine have been reported to increase immediately after reperfusion and reach a peak by 20-40 minutes and 45-75 minutes, respectively. In cardiac operations, it has been shown that the serum levels reached a within one hour after aortic declamping and the levels correlated with the cross-clamp time or the postoperative catecholamine requirements.[6 7] The present study also have demonstrated that the serum H-FABP levels in the control group reached a peak approximately one hour after aortic declamping, which was the earliest among the biochemical markers measured in this study. The peak levels correlated with the peak levels of CK-MB and troponin-T in the control group. Thus, the results of our study and previous studies[6 7] indicate that measurements of serum H-FABP allow for earlier evaluation of myocardial damage among the biochemical markers in cardiac surgical patients with preserved renal function. However, more frequent and earlier blood sampling than what we used may be required to determine the peak value of serum H-FABP because of its rapid clearance from the circulation.

As techniques of operative and postoperative patient management improve, with resulting satisfactory clinical outcome, the population of cardiac surgical patients with preoperative risks, such as advanced age, impaired organ function and repeated heart surgery, have been increasing.[12] Of these preoperative risk factors, renal dysfunction has been reported as an important risk factor for operative mortality.[12-14] Percentages of the patients with preoperative renal dysfunction, defined as either serum creatinine level of greater than 1.9 mg/dL or creatinine clearance of less than 40 mL/min were reported 3.5%[13] and 3.7%,[14] respectively. It has been also reported that the prevalence of acute renal failure after cardiac surgery ranges from 1.1% to 7%,[14 15] depending on the criteria used to determine the complication. Thus, the perioperative management of patients with impaired renal function is still one of the major concerns in cardiac operations. Although serum H-FABP is being accepted as a useful indicator of myocardial damage, some problems may arise when encountering patients with renal insufficiency. In coronary occlusion and reperfusion induced myocardial injury dog model, Sohmia *et al.*[9] have shown that urinary H-FABP increased rapidly after reperfusion and the amount closely correlated with infarct size. In the report, whole-body autoradiography using ^{125}I-labeled H-FABP has also demonstrated that the radioactivity was highly concentrated in the renal cortex and the bladder. We have also demonstrated previously a rapid appearance of H-FABP in urine after reperfusion and a significant inverse correlation between the postoperative urinary H-FABP levels and left ventricular function in patients underwent coronary artery bypass grafting.[16] Thus, H-FABP may be cleared considerably

from kidney,[9 17] similar to that found for myoglobin.[8 18] Therefore, the possibility exists that any change in renal function affects serum or urinary H-FABP concentration, and thus leads to erroneous interpretation. Kleine *et al.*[19] reported that the serum H-FABP level remained increased until 25 hours after the acute myocardial infarction in a patient with severe renal insufficiency, whereas the levels normalized in 10 hours in patients with normal renal function. Gorski *et al.*[20] also have demonstrated that mean serum concentration of H-FABP in patients with chronic renal failure and normal cardiac function was 21 times higher than that in patients with normal renal function. They have also shown that serum H-FABP concentration is much higher (20-25 fold) than that of myoglobin (four fold) despite of similar molecular masses (15 kDa and 18 kDa, respectively). Thus, they suggested that the kidneys play a more dominant role in the clearance of serum H-FABP than of myoglobin. To date, however, it is unknown whether renal function affects the serum levels of H-FABP in patients undergoing cardiac surgery. Our study clearly demonstrated that the serum H-FABP levels were significantly higher in patients with impaired renal function preoperatively, whereas the CK-MB and troponin-T levels did not differ between the two groups. The increase in serum H-FABP levels persisted until 18 hours after reperfusion in renal insufficiency patients, whereas the level peaked approximately our hour and normalized by 18 hours after reperfusion in patients with preserved renal function. Moreover, preoperative creatinine clearance correlated inversely with serum H-FABP levels and correlated positively with urinary H-FABP excretion rate. These findings lend support to the previous results[9 17] that kidneys play a central role in elimination of H-FABP from the circulation. Therefore, it seems very likely that the serum level leads to an overestimation and the urinary level leads to an underestimation of myocardial damage in patients with decreased renal function. To estimate properly the extent of myocardial injury in such patients, one has to take into account not only H-FABP levels but also other biochemical markers, such as CK-MB and troponins. Although serum H-FABP is being accepted as a useful indicator of myocardial damage during cardiac surgery, caution must be taken when using this marker in patients with renal dysfunction.

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